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The potential evolutionary impact of invasive balloon vines on native soapberry bugs in South Africa

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Abstract

Following their establishment in new communities, invasive species may cause evolutionary changes in resident native species. This is clearly true for phytophagous insects, which may adapt rapidly when utilising abundant and widespread introduced hosts. The balloon vines *Cardiospermum halicacabum* and *C. grandiflorum* were introduced to South Africa approximately 100 years ago and are classified as minor and major weeds, respectively. Here we assess the potential evolutionary impact of these vines on native *Leptocoris* soapberry bug populations in Kruger National Park (KNP), using phylogenetic and morphometric analyses. We found that soapberry bugs associated with *C. halicacabum* are genetically and morphologically distinct from those associated with *C. grandiflorum*. This suggests that native soapberry bugs in KNP exhibit some degree of host preference, indicating that these vines may have had significant evolutionary consequences for these insects. The proboscis length of soapberry bugs feeding on *C. halicacabum* closely matched fruit size, often being longer than fruit size at the population level. These soapberry bugs are therefore well-suited to feeding on this introduced plant species.

Keywords

Balloon vine, *Cardiospermum*, invasive species, Kruger National Park, *Leptocoris*, rapid evolution, soapberry bug

Introduction

Evolved and plastic changes are important not only for the colonization and spread of non-native species but are also prevalent in native species as they respond to the presence of introduced taxa (Mooney and Cleland 2001; Strauss et al. 2006; Ghalambor et al. 2007). Common examples of rapid evolutionary change in native species in response to non-native species come from herbivores, particularly phytophagous insects, adapting to introduced plants as preferred hosts (Carroll 2007). For example, evidence for this comes from the study of soapberry bugs (Hemiptera: Rhopalidae), which are specialised predators of seed from the Sapindaceae family (Carroll and Loye 1987). Soapberry bugs feed on the seeds of Sapindaceae species using their elongated, needle-like proboscides to pierce fruits and reach the seeds they contain (Carroll and Loye 1987; Carroll and Boyd 1992). Native soapberry bugs in North America and Australia have colonised several introduced Sapindaceae species (Carroll and Loye 2012). These bugs have subsequently undergone significant adaptive changes in the length of their proboscides and other allometries, directly related to the fruit size of the introduced hosts they utilise (Carroll and Boyd 1992; Carroll et al. 2005a). These changes are heritable and adaptive, facilitating greater feeding efficiency and reproductive success on their new hosts (Carroll et al. 2005a) and have occurred over just 20–50 years (about 40–150 bug generations), indicating the significant selection pressure exerted by these introduced hosts on their newly acquired soapberry predators (Carroll and Boyd 1992; Carroll et al. 2005a). Ultimately, such evolutionary responses in native species could alter contemporary community dynamics and the assembly of future communities, even leading to incipient speciation between native subpopulations (Strauss et al. 2006; Carroll 2007; Andres et al. 2013). Importantly for biological invasions, the rapid evolution of native taxa in response to invaders may impede an invader's spread (Carroll 2011).

The genus *Cardiospermum* L. (Sapindaceae), commonly known as balloon vines, consists of 17 mainly Neotropical species (Gildenhuys et al. 2013). Three species, *C. grandiflorum* Sw., *C. halicacabum* L., and *C. corindum* L., have near-cosmopolitan distributions, although their provenance as native or introduced species remains unresolved in some regions (Gildenhuys et al. 2013). Gildenhuys et al. (2014) showed that *C. corindum* is native to both southern Africa and South America, and confirmed that *C. grandiflorum* and *C. halicacabum* are modern arrivals in southern Africa. *Cardiospermum* species are considered invasive in many parts of the world, often as an unplanned result of intentional introductions for ornamental and medicinal purposes (Carroll et al. 2005b; Simelane et al. 2011; Gildenhuys et al. 2013). Invasive *Cardiospermum* species are commonly classified as 'transformer' weeds, as they often cover native vegetation, potentially driving local biodiversity loss (Henderson 2001; Mc Kay et al. 2010). *Cardiospermum grandiflorum* and *C. halicacabum* were introduced to South Africa approximately 100 years ago (Simelane et al. 2011; Gildenhuys et al. 2013) and are currently listed as Category 1b and Category 3 invaders, respectively, under the National Environmental Management: Biodiversity Act, 2004 (Act No. 10 of 2004). Category 1b species 'may not be owned, imported into South Africa, grown, moved, sold, given as a gift or

dumped in a waterway'. These species are major invaders requiring containment and removal, often with the assistance of government sponsored programs (Alien and Invasive Species Regulations 2014; https://www.environment.gov.za). Category 3 species may remain in certain areas/provinces, but further propagation or trade is prohibited. In South Africa, *C. grandiflorum* is found along most of the Kwa-Zulu Natal coast and in the Gauteng, Limpopo and Mpumalanga provinces (Henderson 2001). *Cardiospermum halicacabum* is less widely distributed in South Africa, largely restricted to the Limpopo and Mpumalanga provinces (Henderson 2001). The high level of specialisation and co-occurrence of soapberry bugs with *Cardiospermum* suggests that soapberry bugs may play an important role in regulating the reproduction of *Cardiospermum* globally (Gildenhuys et al. 2013). For example, seed predation levels by soapberry bugs on native *C. corindum* in Florida can be as high as 90 % (Carroll 1988; Carroll et al. 2003).

Two soapberry bug genera are native to southern Africa, namely *Leptocoris* and *Boisea*, the former being more widely distributed (Göllner-Scheiding 1980, 1997; Carroll and Loye 2012). Twenty-one *Leptocoris* species are endemic to Africa, in association with native Paullinieae and Thouinieae hosts (Carroll and Loye 2012). In South Africa, native *Allophylus* species are common hosts, but native soapberry bugs have also colonised *C. grandiflorum* and *C. halicacabum* in many parts of the country (Carroll and Loye 2012; JF *pers. obs.*). The *Cardiospermum*-soapberry bug system is well-studied in North America and Australia but remains little-explored in the South African context.

In this study, we used phylogenetic analyses, in combination with morphometric measurements, to investigate the potential evolutionary impact of invasive *C. halicacabum* and *C. grandiflorum* on native soapberry bugs (genus *Leptocoris*) in South Africa. To examine whether any evolved differentiation might have resulted from contemporary natural selection, the proboscis lengths of *Leptocoris mutilatus* Gers. were measured with respect to fruit size variation in invasive *C. halicacabum* populations. The expectation was that soapberry bug proboscis lengths will closely match fruit size in *C. halicacabum* and that any shifts in proboscis lengths will correspond to variation in seed capsule (balloon) size in *C. halicacabum* populations.

Methods

Sampling

Thirteen populations of *C. halicacabum* and a single *C. grandiflorum* population were identified in Kruger National Park (KNP; Suppl. material 1: Table S1). Only one population of *C. grandiflorum* was surveyed as local eradication programmes made it difficult to locate more populations in KNP. *Cardiospermum halicacabum* populations were approximately one kilometre apart and the *C. grandiflorum* population was 21.5 km from the nearest *C. halicacabum* population. Adult soapberry bugs present on individuals in these populations were collected and preserved in 70% ethanol. The initial field classification of all collected soapberry bugs was *Leptocoris mutilatus*.

Leptocoris mutilatus is a typical soapberry bug, about 11–16 mm in length, characterised by an overall scarlet red or brownish red colour and a black, bulged head (Göllner-Scheiding 1980). The species has a wide native range distribution, including Madagascar and central, eastern and southern Africa (Göllner-Scheiding 1980).

Leptocoris phylogeny

To confirm the putative *Leptocoris mutilatus* species assignment of soapberry bugs feeding on invasive *Cardiospermum* in KNP, individuals collected from *C. halicacabum* and *C. grandiflorum* populations were selected for phylogenetic analyses (Suppl. material 1: Table S2). An individual bug from five *C. halicacabum* populations and five individuals from the *C. grandiflorum* population were included in these analyses (Suppl. material 1: Table S2). For those bugs associated with *C. halicacabum*, individuals were selected from populations at different distances from one another to minimise potential isolation by distance effects. Reference specimens of six African species and a single Asian *Leptocoris* species were included in the phylogenetic analyses (Suppl. material 1: Table S2). Bug specimens were preserved in 70% ethanol at -80 °C. Genomic DNA was extracted from legs or whole bodies using the DNeasy Blood and Tissue Kit (Qiagen, supplied by WhiteHead Scientific, Cape Town, South Africa) following the manufacturer's instructions. DNA quality and quantity were assessed using NanoDrop ND-1000.

The mitochondrial cytochrome c oxidase subunit I (*COI*) gene was amplified using the universal primers LCOI-1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') and HCOI-2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') (Folmer et al. 1994). Each 30µl reaction contained approximately 100 ng of genomic DNA, 0.2 mM of each dNTP (Fermentas, Thermo Fisher Scientific, Waltham, Massachusetts, United States), 0.5 µM of each primer, 1 U Taq DNA polymerase [Supertherm, Separation Scientific SA (Pty) Ltd, Roodepoort, South Africa], 1 × PCR reaction buffer, 2 mM MgCl2 and 0.2 mg/ml BSA (Promega). PCR cycles consisted of initial denaturation at 95 °C for 5 min, 45 cycles of denaturation at 94 °C for 1 min, annealing at 45 °C for 1 min and elongation at 72 °C for 1 min, and final extension at 72 °C for 30 min.

All PCR products were purified using the QIAquick PCR Purification Kit (Qiagen, supplied by Whitehead Scientific, Cape Town, South Africa). Purified products were sequenced using an ABI 3730 XL automated machine (Central Analytical Facilities, Stellenbosch University, South Africa).

DNA sequence data were aligned using CLUSTALW version 2.1 (Thompson et al. 2003), followed by manual editing in BIOEDIT version 7.0.5.3 (Hall 1999). The final *COI* dataset consisted of 26 accessions: 10 putative *L. mutilatus* from KNP (five from *C. halicacabum* and five from *C. grandiflorum*), two reference specimens of *L. mutilatus*, two putative *L. amictus*, seven putative *L. hexophtalmus*, one *L. productus*, two *L. aethiops* and two *L. vicinus* (Suppl. material 1: Table S2). Two outgroup sequences of *Boisea trivittata* (JX629056.1 and JX629057.1), a species in the same subfamily as *Leptocoris*

(Serinethinae), were obtained from GenBank (https://www.ncbi.nlm.nih.gov/genbank/) and used as outgroup taxa. Species identifications were performed with reference to Göllner-Scheiding (1980, 1997).

A phylogeny was reconstructed using Bayesian inference (BI) and maximum likelihood (ML) approaches. Bayesian inference was conducted using MRBAYES version 3.2.6 (Ronquist et al. 2012) through the CIPRES Science Gateway version 3.3 (Miller et al. 2010). The best-fit DNA substitution model was identified in JMODELTEST version 2.1.6 (Darriba et al. 2012) using Akaike's information criterion (AIC) (Akaike 1974). The chosen DNA substitution model for the *COI* locus was TPM2uf + I + Γ (-1nL = 1816.41, AIC = 6021.58) (Akaike 1974). The base frequencies were as follows, A = 30.10 %, C = 16.22 %, G = 14.56 % and T = 39.12 %. The rate matrix was R(a) [A-C] = R(c) [A-T] = 1.63, R(b) [A-G] = R(e) [C-T] = 10.96, R(d) [C-G] = R(f) [G-T] = 1.00, and the proportion of invariable sites (I) was 0.6540, and the gamma shaped distribution (Γ) was 1.6170. Each model was run for 10 million generations, sampling every 1000 generations using the default parameters. Consensus trees were built after discarding 20% of trees as burn-in and posterior probabilities (*PP*) were estimated based on the percentage of time spent on node recovery. Posterior probability values of < 0.90 were regarded as poor support.

Maximum likelihood analysis was conducted using GARLI version 2.01 (Zwickl 2006) through the CIPRES Science Gateway version 3.3 (Miller et al. 2010). Bootstrap analysis was used to generate branch support values (1000 pseudo-replicates) (Felsenstein 1985). Bootstrap values of < 75% were regarded as indicating poor support. Tree search analysis was performed using a heuristic search algorithm starting at a random tree. Base frequencies were estimated, and four rate categories were included for gamma. Final bootstrap values were discerned via a 50% majority rule tree, generated using PAUP version 4.0 (Swofford 2002).

Allometry of native *Leptocoris* and trait-matching with invasive *Cardiospermum* populations

To investigate whether soapberry bug proboscis lengths track fruit size variation in *C. halicacabum* populations, a total of 311 full-sized fruit and 154 associated soapberry bug individuals were measured from 13 *C. halicacabum* populations in KNP. 20–25 fruit and 5–16 soapberry bugs were measured for each population (Suppl. material 1: Table S3). To compare allometries between bugs found on *C. halicacabum* and those found on *C. grandiflorum*, a further 30 soapberry bug individuals from the single *C. grandiflorum* population were measured. Following Carroll and Loye (1987), the proboscis length of each bug was measured from the anterior tip of the clypeus to the distal tip of the proboscis. Thorax width and body length, taken from the anterior tip of the clypeus to the distal tip of the abdomen, were measured as proxies for body size. For the fruit measurements a cross-section was made just above the seeds using a pair of sharp scissors (Figure 1). Extra care was taken not to deform or compromise

Figure 1. Interior of fruiting capsule of balloon vine *C. halicacabum.* The upper portion of the capsule wall has been trimmed away and shows the central position of the seeds. The 'fruit size' variable we measured was the shortest distance from the fruit capsule perimeter to the seed coat for each seed. The second species in this study, *Cardiospermum grandiflorum*, has larger fruits of similar architecture (Image source: Wikimedia commons: *Cardiospermum halicacabum*).

the integrity of the membranes holding the seeds in place. Fruit size was measured as the shortest distance from the fruit capsule perimeter to the seed coat and repeated for every seed inside each fruit. A further 14 fruits were measured from the single *C. grandiflorum* population in KNP. Bug and fruit measurements were taken using handheld digital callipers with a 0.01 mm resolution.

ANCOVA models were used to quantify and compare proboscis-body size allometries between bugs found on different *Cardiospermum* hosts. For those bugs found on *C. halicacabum*, regression analysis was used to determine whether there was a significant trait-matching relationship between mean population proboscis lengths and mean population fruit sizes. A two-sample *t*-test was used to determine whether mean proboscis length was significantly different between bugs found on *C. grandiflorum* and *C. halicacabum*. A Kruskal-Wallis rank sum test and post-hoc Dunn's test were used to compared proboscis lengths at the population level. Two-sample *t*-tests were also used to determine whether fruit size was significantly different between balloon vine species. Furthermore, two-sample *t*-tests were used to determine the nature and direction of the morphological fit between the fruit sizes of each *Cardiospermum* host and the proboscis lengths of their associated soapberry bug predators.

All statistical analyses were conducted in R (R Core Team 2018).

Results

Sequence variation

The aligned *COI* dataset contained 545 base pairs. All sequences were deposited into the GenBank online repository.

Leptocoris phylogeny

The phylogeny recovered well-supported basal clades for *Leptocoris vicinus* and *L. aethiops*, and a larger clade containing *L. amictus* and *L. hexophtalmus* (Figure 2). All KNP soapberry bugs fell into a monophyletic clade with the representative *L. mutilatus* specimens, sister to *L. productus* (*PP* = 0.99; Figure 2). However, while *Leptocoris* specimens collected from *C. grandiflorum* in KNP grouped unequivocally with *L. mutilatus* reference specimens, all the soapberry bugs collected from *C. halicacabum* fell into a single clade (*PP* = 0.99) sister to the clade containing specimens from *C. grandiflorum* and *L. mutilatus* reference specimens (Figure 2). This suggests that while those bugs feeding on *C. halicacabum* are likely *L. mutilatus*, they are genetically differentiated from those feeding on *C. grandiflorum*. Based on this differentiation, soapberry bugs found on *C. halicacabum* in KNP will hereafter be referred to as 'halicacabum bugs' and those on *C. grandiflorum* as 'grandiflorum bugs'. The ML analysis produced a near-identical tree topology to the BI analysis, but nodal support was generally weaker (Figure 2).

Allometry of native *Leptocoris mutilatus* and trait-matching with invasive *Cardiospermum* populations

There was a significant positive relationship between proboscis length and thorax width for both halicacabum ($p < 0.001$; Table 1) and grandiflorum bugs ($p < 0.01$; Table 1; Figure 3). Likewise, there was a significant positive relationship between proboscis length and body length in halicacabum bugs ($p < 0.001$; Table 1; Figure 4). No significant relationship was found between proboscis length and body length in grandiflorum bugs ($p > 0.05$; Figure 4). Halicacabum bugs had a significantly stronger relationship between proboscis length and thorax width than grandiflorum bugs ($F_{1,180} = 17.03$, $p < 0.001$; Table 1; Figure 3). Similarly, halicacabum bugs had a significantly stronger relationship between proboscis length and body length than grandiflorum bugs ($F_{1,180} = 15.07$, $p < 0.001$; Table 1; Figure 4). However, at the population level, only six halicacabum bug populations showed a significantly stronger relationship for both the proboscis-thorax width and proboscis-body length allometry compared to the grandiflorum bug population ($p < 0.05$; Suppl. material 1: Table S4). Three halicacabum bug populations did not have significantly stronger relationships for both the proboscis-thorax width and proboscis-body length allometry compared to the grandiflorum bug population ($p >$

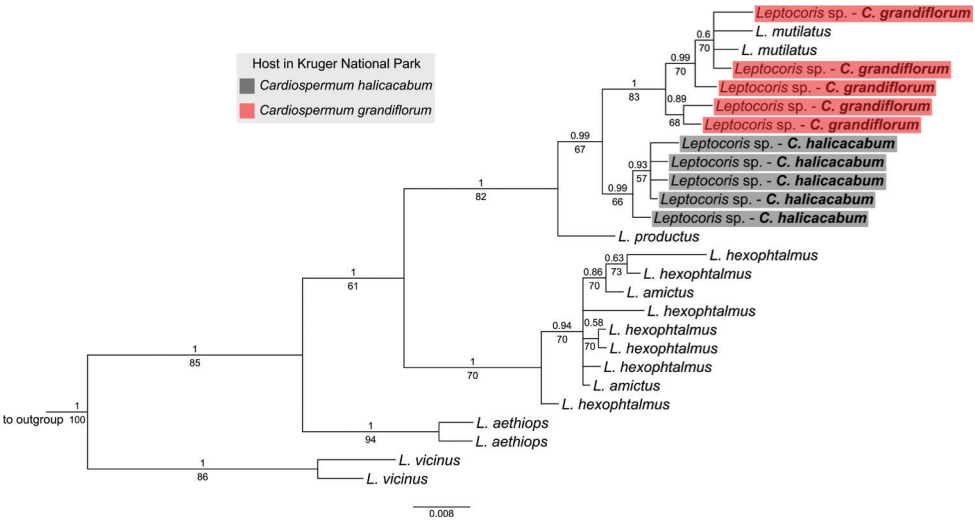

Figure 2. Bayesian phylogeny based on *COI* DNA sequencing data illustrating phylogenetic relationships among African *Leptocoris* species. Shaded branches refer to *Leptocoris* specimens collected from *Cardiospermum halicacabum* and *C. grandiflorum* in Kruger National Park. Nodal support is shown as posterior probabilities and bootstrap values above and below the branches, respectively.

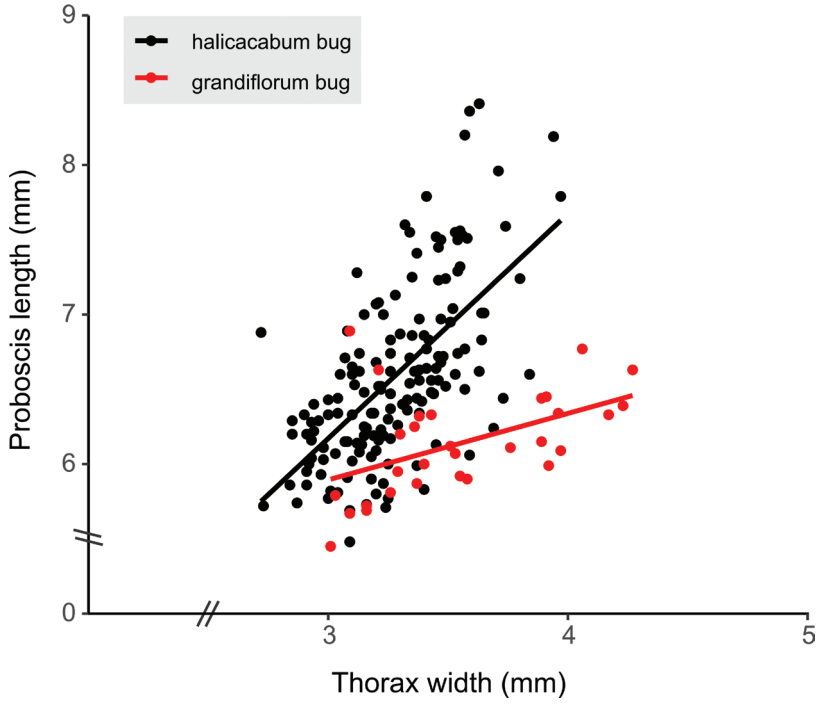

Figure 3. Linear regressions showing the relationship between proboscis length and thorax width for adult *Leptocoris mutilatus* found in association with *Cardiospermum halicacabum* and *C. grandiflorum*. Each point represents an individual.

Table 1. Details of the models used to quantify and compare allometries of soapberry bugs in KNP, and to assess the relationship between population means of halicacabum bug proboscis length (in mm) and *C. halicacabum* fruit size (in mm).

LM (Proboscis length ~ Thorax width * Host plant)				
	Estimate	*SE*	*t*-value	*p*-value
(Intercept)	1.6628	0.4718	3.524	< 0.001
Thorax width	1.5029	0.1436	10.467	< 0.001
Host *C. grandiflorum*	2.8979	0.8949	3.238	< 0.01
Thorax width: Host *C. grandiflorum*	-1.0584	0.2565	-4.126	< 0.001
Residual *SE*: 0.439 on 180 df				
Multiple r^2: 0.4371, Adjusted r^2: 0.4277				
F-statistic: 46.59 on 3 and 180 df, *p*-value: < 0.001				
LM (Proboscis length ~ Body length * Host plant)				
	Estimate	*SE*	*t*-value	*p*-value
(Intercept)	2.1152	0.5319	3.977	< 0.001
Body length	0.3799	0.0451	8.431	< 0.001
Host *C. grandiflorum*	3.0109	0.9499	3.170	< 0.01
Body length: Host *C. grandiflorum*	-0.2989	0.0770	-3.883	< 0.001
Residual *SE*: 0.4734 on 180 DF				
Multiple r^2: 0.3454, Adjusted r^2: 0.3345				
F-statistic: 31.66 on 3 and 180 df, *p*-value: < 0.001				
LM (Mean proboscis length ~ Mean fruit size)				
	Estimate	*SE*	*t*-value	*p*-value
(Intercept)	6.8366	0.7885	8.671	< 0.001
Mean fruit size	-0.0411	0.1262	-0.326	> 0.05
Residual *SE*: 0.1832 on 11 DF				
Multiple r^2: 0.0095, Adjusted r^2: -0.0805				
F-statistic: 0.1062 on 1 and 11 df, *p*-value: > 0.05				

0.05; Suppl. material 1: Table S4). Four halicacabum bug populations had significantly different relationships for one allometry but not the other compared to the grandiflorum bug population (Suppl. material 1: Table S4). The coefficient of variation for proboscis length was 8.95% in halicacabum bugs and 5.59 % in grandiflorum bugs.

There was no significant relationship between population means of *C. halicacabum* fruit size and proboscis lengths of their associated soapberry bug predators ($p > 0.05$; Table 2; Figure 5). Furthermore, there was no significant relationship between population means of the halicacabum bug allometry residuals and *C. halicacabum* fruit size ($p > 0.05$; Suppl. material 1: Table S5). Mean halicacabum bug proboscis length was significantly longer than grandiflorum bugs (Student's *t*-test, $t = 4.00$, df = 182, $p < 0.001$; Table 2). Similarly, proboscis length was significantly different between halicacabum bugs and grandiflorum bugs at the population level (Kruskal-Wallis rank sum test, $\chi^2 = 29.09$, df = 13, $p < 0.01$). However, post-hoc analysis showed only one halicacabum bug population had significantly longer proboscides than grandiflorum bugs, namely population Hali7 (Dunn's test, $z = 3.63$; $p < 0.05$). Fruit size of *C. halicacabum* was significantly smaller than that of *C. grandiflorum* (Student's *t*-test, $t = 6.40$, df = 323, $p < 0.001$; Table 2). However, halicacabum bug proboscis length was significantly

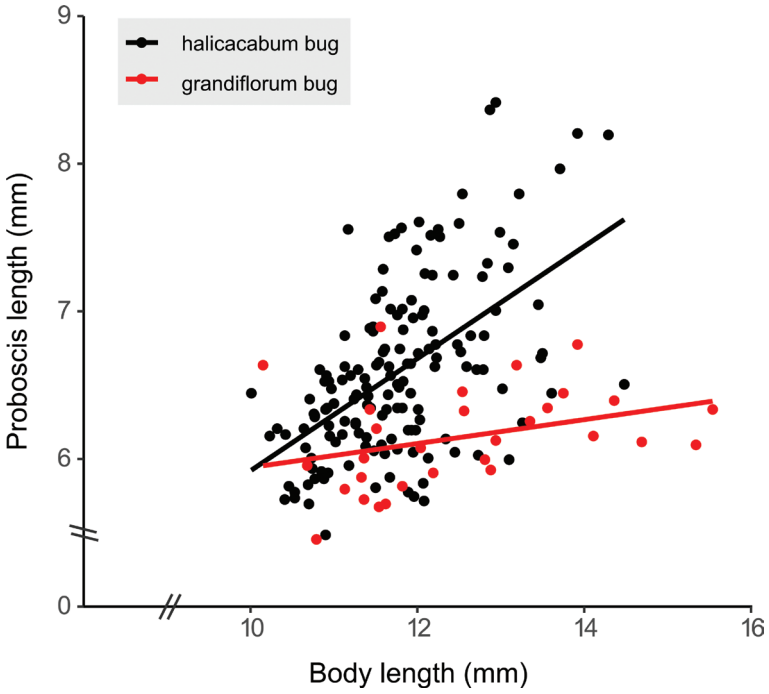

Figure 4. Linear regressions showing the relationship between proboscis length and body length for adult *Leptocoris mutilatus* found in association with *Cardiospermum halicacabum* and *C. grandiflorum*. Each point represents an individual.

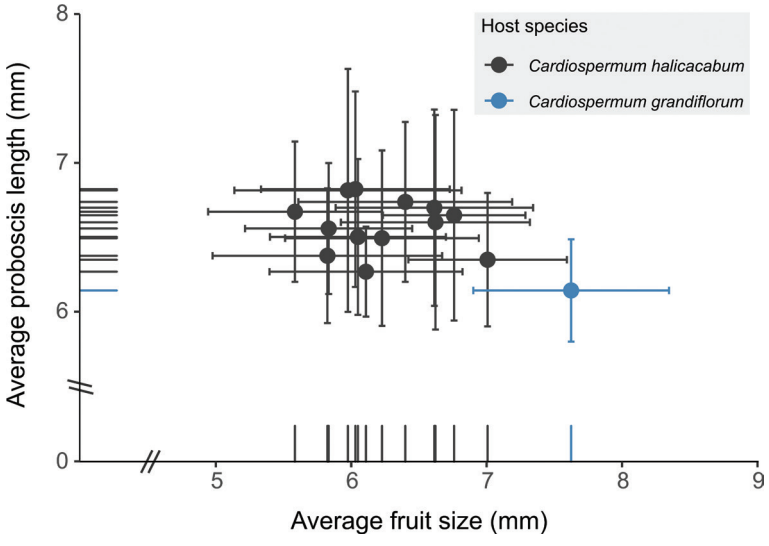

Figure 5. Relationship between proboscis length and fruit size (distance from balloon exterior to nearest seed coat) for soapberry bugs and their associated balloon vine host species in Kruger National Park. Each point represents a single population and error bars are ±SD.

Table 2. Grand means (±SD) of proboscis length and body size measures (in mm) of soapberry bugs in KNP, and fruit sizes (in mm) of their *Cardiospermum* hosts.

Host plant	Proboscis length (mm)	Thorax width (mm)	Body length (mm)	Fruit size (mm)
Cardiospermum halicacabum	6.59 ± 0.59	3.28 ± 0.25	11.77 ± 0.85	6.24 ± 0.80
Cardiospermum grandiflorum	6.14 ± 0.34	3.56 ± 0.38	12.54 ± 1.41	7.62 ± 0.72

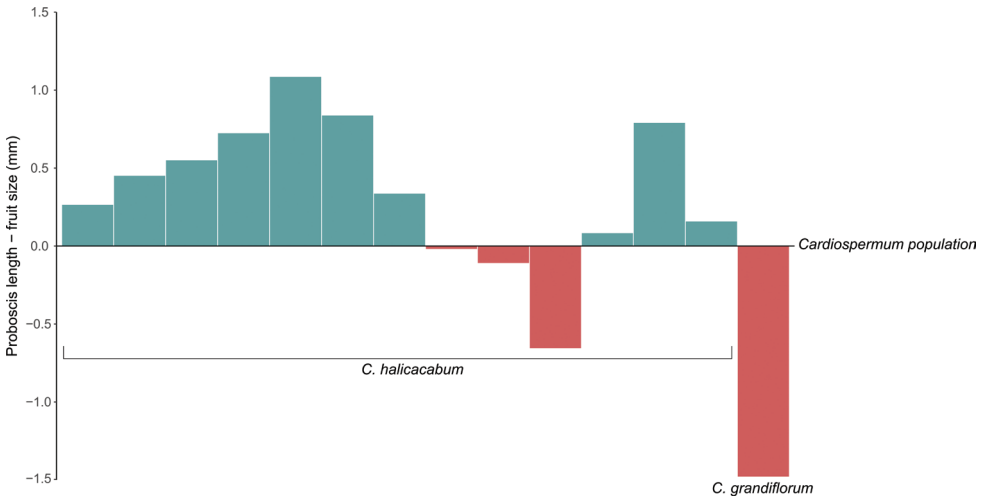

Figure 6. Difference between average proboscis length and average fruit size (i.e. proboscis length - fruit size) for *Leptocoris mutilatus* and *Cardiospermum* populations in KNP. Green bars represent populations in which soapberry bugs are well-suited to feed on their *Cardiospermum* hosts (i.e. proboscis length > fruit size) and red bars represent populations in which soapberry bugs are poorly matched (i.e. proboscis length < fruit size).

longer than the fruit size of their *C. halicacabum* hosts (Student's *t*-test, $t = 4.83$, df = 463, $p < 0.001$; Figure 6, Table 2). In contrast, grandiflorum bug proboscis length was significantly shorter than the fruit size of their *C. grandiflorum* hosts (Welch's *t*-test, $t = 7.29$, df = 15.81, $p < 0.001$; Figure 6, Table 2).

Discussion

This study aimed to assess the potential for impacts by invasive balloon vines on morphological traits of native soapberry bug (genus *Leptocoris*) populations in South Africa's flagship protected area, Kruger National Park (KNP). We found *L. mutilatus* bugs on both invasive host plants and provide some evidence for genetic and morphological differentiation between bugs feeding on different balloon vine species in KNP. Additionally, the proboscis-fruit size trait-matching patterns differed between the two balloon vines. Halicacabum bugs had proboscis lengths well-suited for feeding on the seeds of intact fruits, but this was not the case for grandiflorum bugs.

Genetic differentiation between soapberry bug populations associated with *C. halicacabum* and *C. grandiflorum* in KNP (Figure 2) was mirrored by morphological differentiation between these populations (Figures 3, 4). More specifically, halicacabum bugs had significantly longer proboscides and stronger proboscis length-body size allometries than grandiflorum bugs. This host-associated morphological differentiation agrees with Carroll and Boyd (1992), who found significantly different relationships between proboscis length and body length for another soapberry bug, *Jadera haematoloma*, utilising different native and introduced Sapindaceae species in North America. Similarly, Carroll et al. (2005a) found evidence for a breakdown of proboscis length-body size allometry in an Australian soapberry bug, *Leptocoris tagalicus*. The proboscis length of *L. tagalicus* feeding on introduced *C. grandiflorum* increased relatively more than body size in females and without concomitant body size changes in males compared to native host plant-feeding bugs (Carroll et al. 2005a). Importantly, cross-rearing experiments showed that these changes are heritable, with a strong signal of host-specific genetic differentiation (Carroll et al. 2005a). The moderate host-specific phylogenetic signal we retrieved may point to similar differentiation between halicacabum and grandiflorum bugs in South Africa, i.e. the incipient stages of partial reproductive isolation and therefore 'host race' formation. However, the sampling of a single grandiflorum bug population strongly restricts any inferences made here. In addition to the lack of significant morphological differentiation between halicacabum and grandiflorum bugs for all populations, the amount of variation observed between halicacabum populations suggests that further sampling of bugs from *C. grandiflorum* populations in KNP may reveal overlapping morphological variation between bugs on each host. Therefore, while the genetic and morphological evidence provides some support for incipient host race formation, our sampling bias does not allow us to conclusively reject the possibility that a single variable *L. mutilatus* lineage exploits both invasive balloon vine hosts in KNP. Furthermore, the halicacabum bug populations were geographically distant from the grandiflorum bug population (separated by 21.5 km), suggesting that the genetic differentiation between halicacabum and grandiflorum bugs may be partly explained by isolation by distance and not only host-specific genetic differentiation. Alternatively, the allometries of these soapberry bugs may not be driven by their respective novel hosts at all, but derive rather from distinct ancestral populations with inherently different allometries. Future work should include a wider geographic collection of numerous populations from both native and balloon vine host plants in order to gain an improved understanding of these possibilities.

The lack of a significant proboscis length-fruit size trait-matching relationship across halicacabum bug populations (Figure 5) is perhaps unsurprising considering the limited variation in fruit size between populations (i.e. there is no significant fruit size variation to select for different proboscis lengths in the associated soapberry bug predators). Yet, the match between proboscis length and fruit size is very close (0.35 mm difference in means, Table 2). Importantly, 77% of the halicacabum bug populations had proboscis lengths longer than the average fruit size of their host population (Figure 6). This provides strong evidence that these bugs are well-suited to efficiently

feed on *C. halicacabum* in KNP. Soapberry bugs may therefore have played a role in impeding the spread of *C. halicacabum* in KNP. Future studies should aim to quantify seed predation of introduced Sapindaceae species by soapberry bugs to assess their potential as 'neoclassical' biocontrol (Carroll 2011).

While the fit between proboscis length and fruit size is likely adaptive (Carroll and Boyd 1992, Carroll et al. 2005a), the mechanism behind it should be considered within the plant community context. There is at least one common native sapindaceous species in the immediate study area with fruit sizes that potentially overlap with *C. halicacabum*, namely *Pappea capensis.* This species has large seeds held singly within fruits of 10–15 mm diameter (Palgrave 1992; LF *pers. obs.*) with the distance from the fruit exterior to the seed being ~5mm (SPC *pers. obs.*). In addition, native *Cardiospermum corindum* (Siebert et al. 2010; Gildenhuys et al. 2014) and *Allophylus* species have also been collected from the region (Victor and van Wyk 2005) and could potentially also be sources of colonists. Accordingly, while the fit of halicacabum bug proboscis lengths may result from local adaptation to that host, it could also represent evolution by spatial sorting of migrant genotypes (*sensu* Shine et al. 2011) from surrounding native sapindaceous species with proboscis lengths suitable for feeding on balloon vines. However, considering the small range of proboscis lengths (coefficient of variation = 8.95%) and evidence for introduced balloon vine host specificity by soapberry bugs in other parts of the world (Carroll et al. 2005a; Andres et al. 2013), it is plausible that the proboscis length of halicacabum bugs is under selection by *C. halicacabum* fruit size with allometries different from those feeding on native hosts. Further sampling of soapberry bugs from native sapindaceous hosts in the vicinity of our study area is needed to determine the extent of adaptation by halicacabum bugs.

Interestingly, the average proboscis length of grandiflorum bugs was 1.48 mm shorter than the average fruit size of the associated *C. grandiflorum* population, indicating that these bugs are less well-suited to feed on these fruits. This may imply that these bugs have recently colonised this balloon vine such that selection has not had long to act (Carroll et al. 2005a). It is also possible that no colonisers reaching this larger-fruited species, or their offspring, have had proboscides long enough to reach the encapsulated seeds (*sensu* Cenzer 2018). In that event longer beaks would not have been selected for. Such constrained evolution of access to *C. grandiflorum* seeds could then also help explain the greater relative proboscis lengths (steeper allometries) we observed in halicacabum bugs compared to grandiflorum bugs, which was unexpected considering the larger fruit of *C. grandiflorum*. Nonetheless, three halicacabum bug populations had similarly 'maladapted' proboscis lengths (Figure 6). Therefore, it is plausible that further sampling of grandiflorum bugs would reveal a comparable pattern of variable adaptation to inflated balloon vine seedpods in the region, and it is indeed intriguing that some halicacabum bugs have suitably long beaks to access seeds in some *C. grandiflorum* fruits (Figure 5). The ongoing KNP program to eradicate *C. grandiflorum* meant we did not find additional *C. grandiflorum* sites to survey bugs, and we likewise lack morphological data for soapberry bugs feeding on native sapindaceous species in the region. Hence it remains unclear whether halicacabum or

grandiflorum bugs have the more 'ancestral' allometry or whether the relative change is greater in either host race.

Not knowing about the potentially illuminating influences of native Sapindaceae fruit size on the allometries of halicacabum and grandiflorum bugs is a key shortcoming in this study, and any ongoing local patterns of host-associated differentiation inferred here may be changed by plant eradication efforts. Despite these challenges, our findings add support to considerations of the potentially significant evolutionary impact of introduced balloon vines on native soapberry bug populations, and that these impacts may be dissimilar for different balloon vine species. More extensive sampling in South Africa of soapberry bugs from both introduced balloon vine species and native sapindaceous species is needed to determine the degree of morphological and genetic differentiation between invasive- and native-feeding soapberry bug populations. This will provide a more complete assessment of the potential evolutionary impact of introduced balloon vines on soapberry bug populations, and the potential for these bugs as neoclassical biocontrol of *Cardiospermum* invasions.

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Supplementary material 1

Tables S1–S5.

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Data type: measurements

Explanation note: **Table S1.** Sampling localities for *Cardiospermum* populations included in this study. **Table S2.** Locality data for *Leptocoris* species included in this study. **Table S3.** Number of fruit and soapberry bugs measured per population. **Table S4.** Population-level comparisons of proboscis length-body size allometries between halicacabum and grandiflorum bugs using ANCOVA models. **Table S5.** Details of the linear models used to assess the relationship between population means of the halicacabum bug allometry residuals (in mm) and *C. halicacabum* fruit size (in mm).

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